



Osteoimmune Interactions in Inflammatory Bowel Disease: Central Role of Bone Marrow Th17 TNF α Cells in Osteoclastogenesis

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Osteoimmunology is an interdisciplinary research field dedicated to the study of the crosstalk between the immune and bone systems. CD4⁺ T cells are central players in this crosstalk. There is an emerging understanding that CD4⁺ T cells play an important role in the bone marrow (BM) under physiological and pathological conditions and modulate the differentiation of bone-resorbing osteoclasts. However, identification of the mechanisms that maintain CD4⁺ T cells in the BM is still a matter of investigation. This article describes the CD4⁺ T cell populations of the BM and reviews their role as osteoclastogenic population in inflammatory bowel disease.

Keywords: Th17 Cells, osteoclasts, bone marrow, inflammatory bowel diseases, osteoporosis, osteoimmunology

Bone marrow (BM) has long been known to play an important role in the immune system as a central hematopoietic organ. However, its function in T cell-mediated tolerance or immunity remains elusive. In the past, most of immunological studies were focused on T and B cells in the thymus, the lymph nodes, and the spleen considered as the central reservoirs of adaptive immunity. Over time, it became apparent that the immune system has a far more decentralized governing function. Recently, the BM was shown to play several unexpected roles, serving as an important reservoir for memory T cells including pathogenic cells and long-lived plasma cells involved in the maintenance of long-term immunity and pathogenicity.

BONE MARROW RESIDENT CD4⁺ T CELLS

Memory CD4⁺ T cells provide rapid and highly effective protective immunity against previously encountered pathogens and can recognize a wide variety of antigens. The concept of systemic memory consists of two major subsets: central memory T (T_{CM}) cells and effector memory T (T_{EM}) cells. Importantly, this identification was done in the blood (1). T_{CM} cells express the chemokine receptor CCR7 and the vascular addressing L selectin (CD62L), which enables them to migrate from the blood to the lymph nodes. T_{EM} cells express low levels of CCR7 and CD62L but have receptors that allow them to access peripheral tissues as, for example, the E-selectin ligand cutaneous lymphocyte antigen (CLA), which grants them access to the skin, and $\alpha 4\beta 7$, an integrin that allows them access to the gut. These memory T cells are called tissue-resident memory T cells (T_{RM}) (2).

In the BM, T cells represent about 3–8% of total nucleated cells. BM T cells reach the BM from the blood and, after homing to the BM, can move back to the blood to migrate to other lymphoid organs (3). The rules governing cell migration to the BM have been investigated mainly

in the case of hematopoietic stem cells (HSCs) and revealed the dominant role of the CXCR4–CXCL12 axis in this migration. Analysis of CD4⁺ T cells from the BM of normal mice or mice affected with inflammatory bowel diseases (IBDs) showed the presence of both T_{CM} and T_{EM} cells that are characterized by a high expression of the chemokine receptors CXCR4 and CCR6 (4, 5). However, the mechanism of recruitment of these T cells in the BM remains elusive. Moreover, the degree to which memory cells are resident (T_{RM}) within the BM versus transiting through it is less clear.

In order for T_{RM} cells to be maintained in the BM, they must adapt to the local environment and ignore signals that may induce their egress outside of the BM. The mechanisms and cells involved in the maintenance of BM T cells represent a matter of active research. BM stroma includes mesenchymal stromal cells (MSCs), endothelial cells, osteoblasts (OBLs), and adipocytes. This stromal cell heterogeneity complicates the understanding of the implication of these cells in the maintenance of immunological memory. It is well recognized that BM stromal cells support hematopoiesis by establishing specialized niches. These niches regulate the fate of HSCs in terms of quiescence, migration, and differentiation (6). The major components of the HSC niches include several MSC populations [CXCL12-abundant reticular (CAR) cells and Nestin⁺ cells] (7), OBLs (8, 9), and endothelial cells (10, 11). In addition, regulatory T cells (12), macrophages (13), and osteoclasts (OCLs) (14, 15) were shown to contribute to the regulation of the HSC niches. MSCs have also been involved in the retention of T cells in the BM. Tokoyoda et al. showed that memory CD4⁺ T cells are located close to BM stromal cells expressing IL-7 and VCAM1 (16). This was confirmed by Nemoto et al. who reported that in IBD, pathogenic CD4⁺ T cells are retained in the BM through interactions with IL-7-producing MSCs (17). However, the exact nature of the VCAM1⁺ and IL-7⁺ stromal cells and their role in the maintaining of memory CD4⁺ T cells remain to be elucidated. Adoptive transfer of B and T lymphocytes in mice led to seeding of dendritic cell (DC) clusters with grafted cells in perivascular domains, which were referred to as BM immune niches (18). Overall, these observations suggested the existence of CD4⁺ T cell niches that remain to be characterized in term of cell composition and regulation. The identification of BM immune niches raises many new questions. Which molecules regulate T cell migration? How are memory CD4⁺ T cells maintained and for how long? How do they interact with BM stroma under physiological and inflammatory conditions? The identification of BM-specific factors that control T cell functions is likely to have a major impact on translational medicine.

CROSSTALK BETWEEN CD4⁺ T CELLS AND BONE CELLS

The crosstalk between the immune and bone systems led to the emergence of an interdisciplinary field called osteoimmunology (19). This field emerged from clinical observations reporting that patients presenting an overactivation of the immune system, such as chronic infections and chronic inflammatory diseases, also display bone loss (20–22). The importance of this crosstalk was

further confirmed with the identification of the role of activated CD4⁺ T cells in pathological osteoclastogenesis (23). Its scope has been extended to encompass a wide range of molecular and cellular interactions, including those between immune cells and bone cells, and between bone cells and hematopoietic cells. These interactions are essential for the development of the immune and bone systems (15, 24).

Bone remodeling is a highly regulated process involving complex interactions between the activity of the bone-forming OBLs and the activity of the bone-resorbing OCLs. OCLs are multinucleated cells from the myeloid lineage (monocytes and DCs) (25) that degrade the bone matrix and release growth factors that contribute to the coupling between OCLs and OBLs (26). The mesenchymal-derived OBLs migrate to the eroded sites and initiate new bone formation by the secretion of an extracellular matrix and its calcification. In a physiological state, bone remodeling provides an adequate environment for the development of the immune system and the protection of HSCs (6).

The BM represents a reservoir for memory T cells among which 25% are Foxp3⁺ regulatory T cells (27). It is also a preferential site for the migration or the selective retention and function of regulatory T cells. This finding provides evidence for an unidentified role of the BM in T-cell homeostasis. Moreover, Tokoyoda et al. have shown that in adult mice, more than 80% of antigen specific memory CD4⁺ T cells rest in the BM for more than 90 days after immunization and do not proliferate (16). From these observations, we can hypothesize that the immunosuppressive activity of BM regulatory T cells participates in the quiescence of memory T cells as recently shown for CD8⁺ memory T cells (28). In this study, regulatory T cells suppress proliferation and effector programs during the memory differentiation of CD8⁺ T cells in the lymph nodes (28). In addition to regulatory T cells, MSCs are also generally considered as immunosuppressive cells. MSCs may suppress T-lymphocyte proliferation and functions both *in vitro* and *in vivo* (29, 30). They produce soluble factors, including TGF- β , able to mediate suppression of T-cell proliferation (31). MSCs can also inhibit T cell proliferation by increasing IL-10 secretion (31). These properties suggest that the immunosuppressive effect of MSCs may participate to the preservation of CD4⁺ memory T cells in the BM. However, MSCs are a heterogeneous population of cells and their immune suppressive activity has mainly been explored in pathological conditions. It is therefore important to better understand this function to clarify how MSCs may control the quiescence and niche of CD4⁺ T cells.

To date, the role of CD4⁺ regulatory T cells and memory T cells on bone cells in physiological conditions remains controversial. It has been reported that Rag1^{-/-} mice lacking T cells have a normal bone phenotype (32), whereas T cell-deficient nude mice display, with age, an increased bone resorption and a decreased bone mineral density (33). T cells are capable of mediating anti-osteoclastogenic signals, as depletion of CD4 and CD8 T lymphocytes in mice *in vivo* enhances vitamin D3-stimulated OCL formation by blocking the production of osteoprotegerin (OPG) by B cells (34). These observations revealed the difficulty to conclude on the role of CD4⁺ T cells in steady state osteoclastogenesis because they can have direct and indirect effects on

OCL precursors and also because of their heterogeneity. Indeed, Th1, Th2, and Th17 cells have been reported to have opposite effects on OCL differentiation *in vitro*. Th1 and Th2 cells inhibit OCL formation through their production of INF- γ and IL-4, respectively, whereas Th17 cells have an osteoclastogenic helper effect *in vitro* mediated by MSCs (35).

OSTEOIMMUNOLOGY INTERACTIONS IN INFLAMMATORY BOWEL DISEASE

Inflammatory bowel diseases are inflammatory diseases that affect the gastrointestinal tract. There are two main clinical forms of IBD: Crohn's disease which affects any part of gastrointestinal tract and ulcerative colitis in which the pathology mostly affects the colonic mucosa (36). Several factors including genetic factors, gut microbiota, and the host immune system have been described to contribute to IBD (36). Moreover, these diseases are often associated with extra-intestinal manifestations, in particular, bone loss. Indeed, more than 40% of patients with IBD also present bone loss that remains a major extra-intestinal cause of morbidity leading to an impaired quality of life and productivity (37). The prevalence of osteopenia and osteoporosis in patients presenting with IBD ranges from 22–77% to 17–41%, respectively, depending on the population location or the study design. According to the WHO, osteoporosis is defined as a systemic skeletal disease that occurs when bone resorption exceeds bone formation. In low-turnover osteoporosis, bone resorption is normal whereas the synthesis of bone tissue is reduced. In contrast, in high-turnover osteoporosis, the activity of OCL is increased. Osteoporosis associated with chronic inflammation usually follows the high-turnover pattern, whereas corticosteroid-induced osteoporosis is usually of the low-turnover pattern (38).

The association between chronic inflammation and bone destruction has been recognized a long time ago, leading to the hypothesis of the participation of immune cells in the control of bone remodeling (19). A seminal work published in 1999 by Kong et al. established the role of CD4⁺ T cells in inflammatory osteoclastogenesis. In this study, the authors demonstrated that activated CD4⁺ T cells produce RANKL and induce the differentiation of OCLs *in vitro* (23). Besides that, *ctla4*^{-/-} mice, in which T cells are spontaneously activated, displayed a severe osteoporosis. Interestingly, transfer of CD4⁺ T cells from *ctla4*^{-/-} mice into lymphocyte-deficient *rag1*^{-/-} mice caused bone destruction that can be reversed through inhibition of RANKL by OPG (23). One additional study confirmed these observations and identified a common causal link between intestinal inflammation and bone loss showing that activated T cells producing RANKL accumulate in the BM during intestinal inflammation (39). Taken together, these studies suggest that pathogenic CD4⁺ T cells present in the BM during IBD are potentially osteoclastogenic.

Using a mouse model of colitis, induced in SCID mice by injection of CD4⁺CD45RB^{high} naive T cells, Nemoto et al. found that a large number of CD4⁺CD44⁺CD62L⁻ memory T cells resides in the BM. Transfer of BM memory CD4⁺ T cells into new recipient SCID mice reproduced colitis. These BM CD4⁺

cells of colitic mice have therefore been described as “colitogenic memory T cells” (5). Importantly, these resident BM CD4⁺ memory T cells are closely associated with IL-7-producing stromal cells, and they cannot induce colitis when transferred into IL-7^{-/-} \times Rag1^{-/-} mice, suggesting that IL-7 plays an essential role in their maintenance or survival in the BM (5). Recently, the same group has demonstrated that BM MSCs are the major source of IL-7 and play a pathological role in IBD by forming the niche for these colitogenic CD4⁺ memory T cells (17). However, their osteoclastogenic effect has not been explored.

Although IBD has traditionally been assumed to be a Th1-dependent disease, there is controversy over the role of Th1 on bone homeostasis. IFN- γ has been shown to directly inhibit osteoclastogenesis by interfering with the RANKL–RANK signaling pathway (40). Moreover, *in vitro* differentiated CD4⁺ Th1 cells have been shown to inhibit OCL formation through their canonical production of IFN- γ (35). In contrast, observations in humans suggested that IFN- γ may promote osteoclastogenesis because it improves bone resorption in osteopetrotic patients treated with IFN- γ (41). IFN- γ is also a physiologic inducer of MHC class II expression by APCs resulting in the activation of T cells that induce bone resorption by their secretion of RANKL and TNF- α (42). Together, these data suggest that IFN- γ inhibits OCL formation through direct targeting of maturing OCLs, while it promotes osteoclastogenesis indirectly by stimulating T cell activation.

Nowadays, it is well known that the inflamed gastrointestinal mucosa of patients with IBD is massively infiltrated with Th17 cells and that Th17-related cytokines are produced in excess (43). Based on this, it is obvious that Th17 cells play an important role in the pathogenesis of IBD, which was previously solely attributed to Th1 cells. However, although a pathogenic role in intestine inflammation has been ascribed to Th17 cells, administration of neutralizing anti-IL-17A antibody to patients with Crohn's disease did not show any therapeutic benefit (44). Moreover, in some patients, it exacerbated the disease suggesting a protective role of IL-17A (44). Thus, despite the role of Th17 cytokines is important in many aspects of intestinal homeostasis and protection from mucosal pathogens, their role in the pathogenesis of IBD remains ambiguous. However, IL-17 represents a potent osteoclastogenic cytokine, and its receptors are expressed by many cell types, including OBLs and OCLs (45). Kotake et al. reported that IL-17 is abundant in rheumatoid synovial fluid and that IL-17 stimulates OCL differentiation by inducing RANKL expression by OBLs (46). It should be noted that the effect of IL-17 is not limited to this direct action on the OBLs. IL-17 facilitates local inflammation by recruiting and activating immune cells, which leads to an abundance of inflammatory cytokines such as TNF- α (46). IL-17-deficient mice are resistant to bone destruction induced by LPS (35). Otherwise, Oostlander et al. suggested a particular role for IL-17 in osteoclastogenesis in Crohn's disease patients (47). More recently, we have shown that BM Th17 T cells expressing high levels of TNF α were able to induce OCL differentiation in IBD mice. This T-cell-induced OCL differentiation could be inhibited by IL-17 blockade (4) suggesting the importance of IL-17A in osteoporosis.

A picture emerged from the literature (48, 49) to define what we believe to be osteoclastogenic T cells: first, osteoclastogenic T cells should not produce a large amount of IFN- γ . Second, they should trigger local inflammation and the production of inflammatory cytokines, including TNF- α , that induce RANK-L expression on MSCs. Third, they should express RANK-L and might directly participate in the increase of osteoclastogenesis.

Recent data indicate that in IBD, Th17 cells-producing TNF- α represent the long-sought-after osteoclastogenic T cell subset that fulfills all the criteria mentioned above (4, 50). In murine models of IBD associated with bone loss, we have shown that Th17-producing TNF- α cells accumulate in the BM, the colon, and spleen and have a potent capacity to induce OCL differentiation without addition of any exogenous osteoclastogenic factors (4). Through their production of RANK-L and TNF α , Th17 cells directly induce the differentiation of precursors into OCLs (4), but they also have a major effect on MSCs through their production of IL-17. Indeed, IL-17 increases RANK-L expression in MSCs leading to increased OCL formation (4, 35, 46). Moreover, in the context of IBD, Th17 cells increase the expression of monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein 1 α (MIP-1 α) by MSCs, which may promote the recruitment of inflammatory monocytes (OCL precursors) in the BM and their differentiation into OCLs (4). To translate these findings into human disease, IL-17-producing T cells from the blood of IBD patients are osteoclastogenic cells *in vitro* and increase MIP-1 α and MCP-1 expression by human MSCs (4, 47), suggesting their participation to osteoporosis in these patients. Therefore, Th17 cells represent a key target for innovative therapeutic approaches for IBD-associated bone destruction.

CONCLUDING REMARKS

Recent advances have contributed to our understanding of the biology of CD4⁺ T cells in the BM. These T cells fulfill both homeostatic and pathological functions with respect to the bone system. IBD is an immune-mediated disease characterized by systemic Th1 and Th17 responses and bone destruction. Recent studies have revealed that Th17 cells are not only required for the initiation of systemic immune response as they are critical regulators in the chronic inflammation associated with bone destruction, particularly in rheumatoid arthritis. Our recent findings extend these observations to IBD in which the site of inflammation

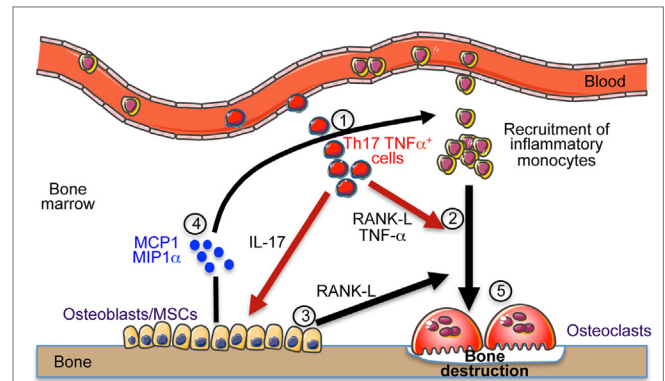


FIGURE 1 | Th17 cells induce bone destruction in IBD. In IBD, Th17 TNF α cells migrate and accumulate in the bone marrow (1). They express RANK-L and TNF- α that participate to osteoclast differentiation (2). They also express IL-17 that stimulates the local inflammation and activates osteoblasts to produce RANK-L inducing osteoclast formation (3) and MCP-1 and MIP-1 α chemokines (4) participating to the recruitment of osteoclast precursor cells (monocytes) in the BM that contribute to the increased osteoclastogenesis (5) and to bone destruction.

is far from the bone and provide the first characterization of osteoclastogenic Th17 TNF- α ⁺ cells in the BM linking IBD and bone destruction.

Collectively, we propose that these osteoclastogenic cells, once in the BM, enable the secretion of chemokines and RANKL by stromal cells. This enhances the recruitment of inflammatory monocytes and DCs that differentiate into OCLs and increase the bone resorption leading to osteoporosis as illustrated in **Figure 1**.

AUTHOR CONTRIBUTIONS

AW wrote and reviewed the manuscript. CB-W and MR reviewed the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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